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PRIMER NOTE

Isolation and characterization of 15 microsatellite loci from mango (*Mangifera indica* L.) and cross-species amplification in closely related taxa

R. J. SCHNELL, C. T. OLANO, W. E. QUINTANILLA and A. W. MEEROW*
**USDA-ARS-National Germplasm Repository, 13601 Old Cutler Rd., Miami, Florida 33158, USA*

Abstract

We report here on the development and characterization of 15 microsatellite loci isolated from *Mangifera indica* L. These markers were evaluated using 59 Florida cultivars and four related species from the USDA germplasm collection for mango. Two loci were monomorphic and 13 polymorphic, with two to seven alleles per locus. Four loci departed significantly from Hardy–Weinberg equilibrium and have significant heterozygote deficiency. Nine loci exhibited significant linkage disequilibrium. Cross-species amplification was successful in four related species. These loci are being used to investigate patterns of genetic variation within *M. indica* and between closely related species.

Keywords: genetic diversity, mango, microsatellite, SSR

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Mangifera indica L. is a minor crop in the USA with 650 ha currently under cultivation in South Florida and 870 ha in Puerto Rico. Genetic relationships among mango cultivars have been studied using randomly amplified polymorphic DNA (RAPD) markers (Schnell *et al*. 1995; Lopez-Valenzuela *et al*. 1997), DNA fingerprinting [DFP (Adato *et al*. 1995)], anchored simple sequence repeat markers (Eiadthong *et al*. 1999) and amplified fragment length polymorphisms [AFLP (Kashkush *et al*. 2001)]. Viruel *et al*. (2005) developed the first reported set of 16 microsatellite markers for mango, of which, 14 produced the expected one or two amplification products per genotype. We have developed an additional set of 15 microsatellite loci and tested these using 59 mango cultivars selected in south Florida and four closely related species: *Mangifera casturi* Kostermans, *Mangifera griffithii* Hook. f., *Mangifera laurina* Blume and *Mangifera odorata* Griff.

An enrichment protocol based on a modified version of the method described by Edwards *et al*. (1996) was used to develop the mango microsatellites. Genomic DNA was extracted from the cultivar 'Haden' and digested with *Sau*3AI. The resulting DNA fragments were ligated to *Sau*3AI linkers (ICBR 1998), and DNA was preamplified using *Sau*-L-A primer. Probes with four dinucleotide, three trinucleotide and three tetranucleotide motifs were used to enrich for fragments containing microsatellites. The 'Haden' genome was subjected to two cycles of enrichment to increase the frequency of microsatellite containing fragments. Size fractionation was performed on the products of a postenrichment amplification using SizeSep 400 Spin Column Sepharose (Amersham Pharmacia Biotech), and the cloning reaction was carried out using the TOPO TA Cloning Kit (Invitrogen) according to manufacturer's recommendations. The plasmid inserts generated from the cloning step were amplified using universal M13 primers and the resulting polymerase chain reaction (PCR) products treated with exonuclease I (Life Technologies). PCR products were sequenced using BigDye Terminator™ Cycle Sequencing Ready Reaction Kit version 2.0 (Applied Biosystems). The cycle sequencing products were analysed on an ABI PRISM 3100 using SEQUENCING ANALYSIS software version 3.7 (Applied Biosystems). Microsatellite containing sequences were imported into GCG (Accelrys) for primer design. Primer pairs were designed for 41 putative microsatellite loci. PCR amplification reactions were carried out in a total volume of 10 μL, containing 0.25 ng/μL genomic DNA. All PCRs contained 0.05 U/μL Ampli*Taq* (Applied Biosystems), 0.2 mM dNTPs, 0.2 μM each forward and reverse primers, 1 mg/ml BSA and 1× GeneAmp PCR buffer [1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 50 mM KCl,

Correspondence: R. J. Schnell, Fax: +01 305 696 6410; E-mail: rschnell@ars-grin.gov

Table 1 Primer sequences and characteristics of 15 *Mangifera indica* microsatellite loci used to analyse 59 Florida cultivars; T_a, annealing temperature for the primer pair; n, number of individuals analysed; H_E, expected heterozygosity; H_O, observed heterozygosity; PIC, polymorphic information content

Locus	GenBank Accession no.	Primers (5′–3′)	Repeat	T_a (°C)	n	No. of alleles	Allele size range (bp)	H_E	H_O	PIC
MiSHRS-1*	AY942817	F: TAACAGCTTTGCTTGCCTCC R: TCCGCCGATAAACATCAGAC	$(CT/AG)_{14}$	50	59	5	191–207	0.664	0.712	0.598¶
MiSHRS-4†	AY942818	F: CCACGAATATCAACTGCTGCC R: TCTGACACTGCTCTTCCACC	$(CT/AG)_{11}$	57	59	5	121–131	0.683	0.661	0.630¶
MiSHRS-18*	AY942819	F: AAACGAGGAAACAGAGCAC R: CAAGTACCTGCTGCAACTAG	$(AAC/GTT)_8$	50	59	6	90–111	0.628	0.610	0.552¶
MiSHRS-23†	AY942820	F: AGGTCTTTTATCTTCGGCCC R: AAACGAAAAAGCAGCCCA	$(TATG/CATA)_7$	65	54	2	199–203	0.466	0.389	0.335
MiSHRS-26‡	AY942821	F: TGTAGTCTCTGTTTGCTTC R: TTCTGTGTCGTCAAACTC	$(GTT/AAC)_6$	57	58	3	260–275	0.229	0.241	0.205¶
MiSHRS-29‡	AY942822	F: CAACTTGGCAACATAGAC R: ATACAGGAATCCAGCTTC	$(TG/CA)_9$	46	59	4	174–182	0.517	0.508	0.460¶
MiSHRS-30*	AY942823	F: AGAATAAAGGGGACACCAGAC R: CCATCATCGCCCACTCAG	$(GTTGTGT/ACACAAC)_3$	51	58	1	222	—	—	—
MiSHRS-32†	AY942824	F: TTGATGCAACTTTCTGCC R: ATGTGATTGTTAGAATGAACTT	$(CA/TG)_9$	53	59	7	200–224	0.387	0.407	0.358
MiSHRS-33†	AY942825	F: CGAGGAAGAGGAAGATTATGAC R: CGAATACCATCCAGCAAAATAC	$(CGG/CCT)_7$	46	59	3	236–248	0.301	0.254	0.263§¶
MiSHRS-34‡	AY942826	F: TGTGAAATGGAAGGTTGAG R: ACAGCAATCGTTGCATTC	$(GTT)_5GCA(GTT)_5$	46	59	1	228	—	—	—
MiSHRS-36*	AY942827	F: GTTTTCATTCTCAAAATGTGTG R: CTTTCATGTTCATAGATGCAA	$(CT/AG)_{15}$	50	51	4	174–190	0.364	0.078	0.331§¶
MiSHRS-37*	AY942828	F: CTCGCATTTCTCGCAGTC R: TCCCTCCATTTAACCCTCC	$(AG/CT)_9$	46	58	4	127–132	0.645	0.707	0.593
MiSHRS-39†	AY942829	F: GAACGAGAAATCGGGAAC R: GCAGCCATTGAATACAGAG	$(GTT/AAC)_8$	53	58	5	348–369	0.597	0.621	0.514
MiSHRS-44‡	AY942830	F: AACCCATCTAGCCAACCC R: TTGACAGTTACCAAACCAGAC	$(TC/GA)_{11}(TA)_{10}$ $(CA/TG)_9(TA)_3(CA/TG)_3$	57	52	2	253–260	0.444	0.231	0.343§¶
MiSHRS-48‡	AY942831	F: TTTACCAAGCTAGGGTCA R: CACTCTTAAACTATTCAACCA	$(GA/TC)_{15}$	57	56	5	201–226	0.701	0.286	0.634§¶

*, labelled with NED fluorescent dye; †, labelled with HEX fluorescent dye; ‡, labelled with 6-FAM fluorescent dye; §, departs significantly from HWE at $P < 0.05$; ¶, linkage disequilibrium.

0.001% (w/v) gelatin]. Thermal cycling profile consisted of the following: 4 min denaturation at 94 °C; followed by 32 cycles of denaturation at 94 °C for 30 s, 1 min at appropriate annealing temperature for each primer (Table 1), 1 min extension at 72 °C; with a final 5 min 72 °C extension. For primer pairs MiSHRS-4, MiSHRS-44 and MiSHRS-48, a touchdown cycling program was used where the annealing temperature was reduced by 1 °C each cycle for 10 cycles to the appropriate annealing temperature, at which temperature, 22 additional cycles were performed. PCR was accomplished on a DNA Engine tetrad thermal cycler (M J Research). Capillary electrophoresis (CE) was performed on either an ABI PRISM 3100 Genetic Analyser or an ABI PRISM 3730 Genetic Analyser (Applied Biosystems).

Primer pairs for 15 loci generated one or two amplification products per genotype, and gave consistent, interpretable results. Forward primers were labelled with fluorescent dyes (Table 1). Preliminary analysis of raw microsatellite data was performed using GENEMAPPER 3.0 (Applied Biosystems). Descriptive statistics (Table 1) were generated with GDA version 1.1 (Lewis & Zaykin 2002). Test for Hardy–Weinberg equilibrium (HWE, exact test) and linkage disequilibrium (LD) were run with GENEPOP version 3.4 (Raymond & Rouset 1995).

The polymorphic information content (PIC) values were low to moderate varying from 0.21 to 0.63 for the polymorphic loci (Table 1). Two of the loci (MiSHRS-30 and MiSHRS-34) were monomorphic in the Florida cultivars, although they are polymorphic in other populations of mango (Schnell RJ unpublished data) and within the four related species. Four loci (MiSHRS-33, MiSHRS-36, MiSHRS-44 and MiSHRS-48) depart significantly from HWE ($P < 0.05$) in this population

and show significant heterozygote deficiency. The cause of this deficiency is unknown and the possibility of null alleles at these loci needs to be investigated. Heterozygote excess was not observed at any locus and nine loci showed significant LD ($P < 0.05$). All 15 primer sets amplified one or two products in each of the four related species. The low number of founder genotypes, unequal distribution of alleles due to selection for horticultural types and clonal propagation are the most likely reasons for the high number of loci in LD (Flint-Garcia *et al*. 2003).

The 14 microsatellites developed by Viruel *et al*. (2005) and the set reported here are being used to investigate genetic diversity in the large mango germplasm collection maintained by the USDA-ARS in the germplasm repository in Miami, Florida and to verify the pedigree of the current Florida commercial cultivars.

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